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The effect of catecholamines and sympathetic stimulation on the release of acetylcholine from the guinea-pig colon

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1. In isolated guinea-pig terminal colon, the effect of sympathetic stimulation on contraction and acetylcholine release elicited by pelvic and transmural stimulation was investigated.

2. Sympathetic stimulation reduced the nerve-mediated contractile responses more than those produced by added acetylcholine.

3. Sympathetic stimulation also reduced the acetylcholine released during pelvic and transmural stimulation at low frequency. The inhibitory effect on acetylcholine released from resting colons is concealed by the simultaneous release of acetylcholine in considerable amounts from stimulated periarterial nerves which probably contain parasympathetic fibres.

4. The inhibitory effect of endogenous and exogenous catecholamines prevails when cholinergic neurones fire at low rates. It was confirmed that adrenaline is more active than noradrenaline.

5. The release of acetylcholine from unstimulated colons was for the most part maintained by nerve-conducted activity, because tetrodotoxin was able to reduce it to about one-tenth.

6. It is suggested that the sympathetic control of gastrointestinal tone and motility is exerted through two different routes: inhibition of intramural cholinergic plexuses and direct relaxation of smooth muscle cells.

7. The possible site and mechanism of action of catecholamines on intramural cholinergic structures is briefly discussed.

The possible modulation of ganglionic transmission by the sympathetic system is still an open question and a matter of discussion. From careful investigations carried out on the gastrointestinal tract of rabbits and guinea-pigs, Gershon (1967) drew the conclusion that the sympathetic system exerts a direct inhibitory action on the smooth muscle without interfering with intramural ganglionic transmission of the parasympathetic outflow. Jansson & Martinson (1966) and Kewenter (1965), however, reached the opposite conclusion by examining the effect of the direct and reflex stimulation of the sympathetic nerves on the vagal control of gastric activity in the cat. Indeed, many morphological and pharmacological observations support the hypothesis that there is a sympathetic control of ganglionic transmission.

Using the histochemical method of Falck, Hillarp, Thieme & Thorpe (1962), Hamberger & Norberg (1965), Jacobowitz (1965) and Äberg & Eränko (1967) demonstrated that most of the sympathetic fibres present in the intestinal wall end in the myenteric and submucosal plexuses, where they make a dense network around non-fluorescent cholinergic cells. Only a few fluorescent fibres reach the longitudinal and circular muscle layers in which some cholinergic fibres are also present. These histochemical findings strengthen the idea that at least a part of the sympathetic control of intestinal tone and motility may be mediated through its modulating effect on the cholinergic intramural structures. Many pharmacological findings suggest that catecholamines interfere with synaptic transmission both in sympathetic and parasympathetic ganglia, including those of gastrointestinal tract (Marrazzi, 1939a, b ; Bülbring & Burn, 1942 ; Marrazzi & Marrazzi, 1947 ; Kewitz & Reinert, 1952 ; Lundberg, 1952 ; McDougal & West, 1954 ; Matthews, 1956 ; Trendelenburg, 1956 ; Kosterlitz & Robinson, 1957 ; Costa, Revzin, Kuntzman & Brodie, 1961 ; Eccles & Libet, 1961 ; Pardo, Cato, Gijon & Alonso-de-Florida, 1963 ; Beleslin & Varagić 1964 ; Elliot, 1965).

According to Trendelenburg (1961) and Kharkevich (1967) the conclusion may be drawn that low doses of catecholamines improve synaptic transmission, while higher ones depress it.

The inhibitory effect is evident at lower rather than at higher frequencies. At high frequencies, in fact, catecholamines seem to counteract presynaptic failure, not only in the ganglia (Elliot, 1965 ; McIsaac, 1966), but also at the neuromuscular junction (Krnjević & Miledi, 1958 ; Bowman, Goldberg & Raper, 1962).

Adrenaline is more potent than noradrenaline (Pardo *et al.*, 1963 ; Matthews, 1956 ; Cairncross, McCulloch, Story & Trinker, 1967) and blockade of α-receptors abolishes their inhibitory action both on transmission (Kewitz & Reinert, 1952 ; Lundberg, 1952 ; Costa *et al.*, 1961 ; Eccles & Libet, 1961 ; De Groat & Volle, 1964 ; McIsaac, 1966) and on acetylcholine release (Schaumann, 1958 ; Vizi, 1968 ; Watt, 1966).

In this work we have tried to demonstrate the inhibitory action exerted not only by catecholamines but also by sympathetic nerves on cholinergic pathways in the guinea-pig distal colon.

Methods

Setting up of the colon

Guinea-pigs of either sex weighing between 300 and 350 g were used. The terminal colon supplied with both sympathetic (periarterial) and parasympathetic (pelvic) nerves as first described by Garry & Gillespie (1955) and Rand & Ridehalgh (1965) was carefully dissected from animals anaesthetized with ether. The preparation, length 4–5 cm, was suspended in a bath containing 6 ml. of oxygenated Tyrode solution at 35° C. Both the oral and aboral ends were left open and the mucous produced during the experiment was removed by suction, if necessary. The oral end was connected to a gimbal writing lever, magnification 1 : 10, load 2·5 g. Pelvic and sympathetic nerves were separately placed in bipolar stimulating electrodes made with rings of silver wire. Stimulation of the intestinal wall (Paton, 1954) was made through a silver wire inserted into the lumen, the reference electrode being placed in the bath. In order to perform the simultaneous stimulation of both

sympathetic and pelvic (or intramural) nerves, rectangular pulses of supramaximal voltage, width 1 msec were delivered separately from two electronic stimulators, supplied with stimulus isolation units. The frequency and the duration of stimulation periods ranged respectively from 1 to 100/sec and from 30 sec to 10 min.

Normal responses to nerve stimulation

In order to establish the possible effect of sympathetic stimulation on the contractile response of the colon elicited by pelvic and transmural stimulation or by exogenous acetylcholine, some preparations were kept in normal Tyrode solution and the nerve-mediated or acetylcholine contractions were compared before and during sympathetic stimulation.

Acetylcholine release

One hour after having set up the colon, the effectiveness of transmural, pelvic and sympathetic stimulation was ascertained with trains of pulses of 1 msec at 20/sec, lasting 30 sec. Then, eserine sulphate 1×10^{-5} g/ml. was added. The drug caused a sustained spasm of the colon so that any contractile response to pelvic or transmural stimulation was abolished; only a relaxation was seen during sympathetic and transmural stimulation. After 40–60 min the preparation was washed with Tyrode plus eserine and the collection periods were started following the procedure previously described (Beani, Bianchi & Ledda, 1964): during the first period, the preparation was stimulated for 1–10 min at the selected frequencies and then left at rest for a further 10 min, at which time the Tyrode solution was removed for bioassay. This first period therefore lasted 11–20 min. After washing, the preparation was kept at rest for 20 min, then the Tyrode solution was removed and re-estimated. The amount of acetylcholine released during this second period was used to calculate the background release in 11–20 min which was subtracted from that released in the first period so as to obtain the actual acetylcholine release associated with the stimulation. The first plus the second period was taken as a trial cycle which was repeated three to five times, either by changing the stimulation schedule or by adding drugs. The acetylcholine content of the samples suitably diluted with distilled water was assayed against acetylcholine standard solutions on eserinized frog rectus abdominis muscle, or on leech dorsal muscle according to Murnaghan (1958). The responses to the samples were abolished after boiling them at pH 10 for 3 min, or in the presence of (+)-tubocurarine 5×10^{-6} g/ml., confirming that the active substance released from the colon was acetylcholine or a similar compound. The presence of interfering substances in the bath fluid was tested and excluded by adding a known amount of acetylcholine to the samples and by repeating the assay.

Estimation of total tissue acetylcholine and cholineacetyltransferase activity

(1) Total tissue acetylcholine was extracted by the method of Beani & Bianchi (1963) from unstimulated terminal colons incubated in Tyrode solution (with or without eserine 1×10^{-5} g/ml. and adrenaline 1×10^{-7} g/ml.); (2) cholineacetyltransferase activity was determined on samples of acetone powder prepared from freshly excised colons by the procedure previously described (Bianchi, Beani & Bolletti, 1966). The reaction mixture suggested by Bull, Hebb & Ratkovic (1963) was used.

In both cases the amounts of acetylcholine (extracted or synthesized) were estimated on eserinized frog rectus abdominis muscle against suitable standards.

Drugs

Phosphotransacetylase (Boëhringer) and commercially available reagents were used to determine cholineacetyltransferase activity. Freshly prepared solutions of acetylcholine chloride, adrenaline and noradrenaline acid tartrate, hexamethonium bromide, eserine sulphate, tetrodotoxin (Sankyo) were used. The concentrations are given as salts. The amounts of acetylcholine released, extracted, or synthesized are expressed as acetylcholine Cl μg/g of fresh tissue.

Results

Normal responses to nerve stimulation

In the colons kept in normal Tyrode solution (without eserine) the height of contraction due to pelvic and transmural stimulation was, within certain limits, directly related to the frequency of pulses. Trains of supramaximal stimuli at 10/sec, lasting 30 sec, gave rise to a response which was about twice that observed with frequencies of 2/sec. So as to obtain submaximal contractions, acetylcholine concentrations ranging from 5×10^{-8} to 2×10^{-7} g/ml. were used. As shown in Table 1, sympathetic stimulation at 50/sec appreciably reduced the responses to pelvic stimulation at every frequency and to transmural stimulation at the lower frequency, but it was ineffective against acetylcholine contractions. This finding is not conclusive for a sympathetic inhibition of the nerve-mediated response: it merely suggests that the functional antagonism, between catecholamines and acetylcholine, in the smooth muscle, is more evident when both transmitters are released into the intestinal wall than when acetylcholine, added to the bath reaches directly the outer muscular layers.

Acetylcholine release during rest and stimulation

Both the release at rest and during stimulation varied appreciably from one group of preparations to another, probably because different lots of animals had to be used in the course of the experiments lasting some months; no clear relationship

TABLE 1. *Effect of sympathetic stimulation at 50/sec on the contractile responses of guinea-pig distal colon elicited by pelvic and transmural stimulation for 30 sec and by acetylcholine*

	Pelvic stimulation			Transmural stimulation			Acetylcholine (g/ml.)		
Experimental condition	2/sec	5/sec	10/sec	2 sec	5/sec	10/sec	5×10^{-8}	1×10^{-7}	2×10^{-7}
Normal responses (mm ± S.E.)	39 ± 7·2	69 ± 7·2	77 ± 7·7	69 ± 6·7	110 ± 5·4	114 ± 5	27 ± 1·8	43 ± 3·6	65 ± 3·3
Responses during sympathetic stimulation (mm ± S.E.)	7·7 ± 2·2†	27 ± 5·4†	48 ± 5·8*	26 ± 4†	94 ± 8	118 ± 5	23 ± 2·5	37 ± 3·1	65 ± 3·1

Each value is the mean of five experiments.
* Significantly different from normal responses, $P < 0.05$, Student's test for paired data.
† $P < 0.01$.

with seasonal or environmental conditions could be found. The average acetylcholine release of 118 unstimulated colons was about 400 ng/g per 10 min and it was constant for at least 3–4 hr. According to Paton & Zar (1968), Ogura, Mori & Watanabe (1966), Zar (1966) and Harry (1962), the greater part of resting release depends on the activity of intramural cholinergic neurones ; as will be shown later, in the guinea-pig colon 90% of the release is linked to conducted nerve activity. It is evident from Fig. 1 that there is a complex relationship between stimulation rate and acetylcholine release. The optimal rate for pelvic nerve stimulation lies between 10–20/sec and for transmural stimulation between 20–60/sec. This difference in the optimal rate may depend on the development of synaptic failure during long-lasting pelvic nerve stimulation at higher frequency. Clearly direct stimulation of the intestinal wall is able to activate a greater number of cholinergic neurones than stimulation of the extrinsic parasympathetic outflow, as judged from the total amount of acetylcholine released.

In every instance, however, there is an inverse relationship between stimulation rate and output/stimulus in agreement with the recent findings of Paton & Zar (1968) (Fig. 2). The ability of intramural cholinergic structures to maintain a sustained acetylcholine release was checked by stimulating the intestinal wall at supramaximal voltage at 10/sec for periods of different duration: 1, 2, 5 and 10 min. As shown in Fig. 3, the total amount of acetylcholine released during the stimulation periods increases in a manner roughly proportional to the duration of stimulation. However, the amount released in the first minute is twice as much as the average release/min during the 10 min period of stimulation. The colon surviving in Tyrode solution is therefore unable to maintain a steady acetylcholine release in the

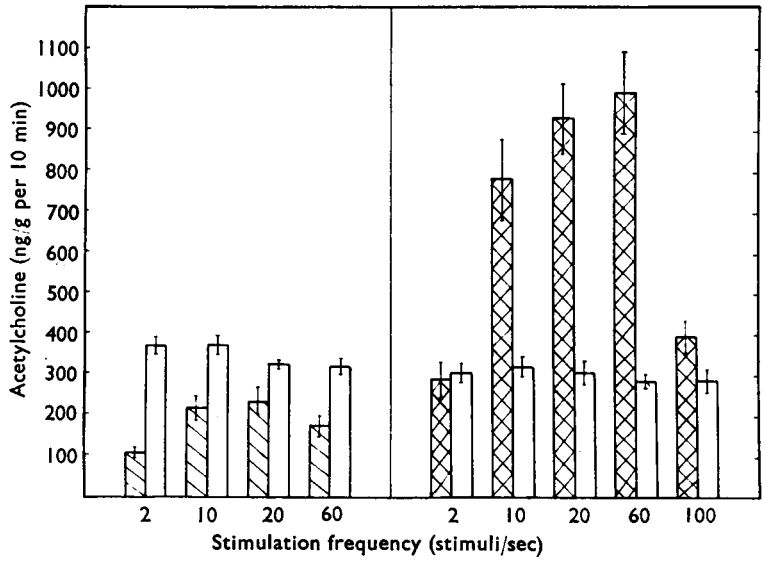

FIG. 1. Acetylcholine release (ng/g per 10 min ± S.E., vertical lines) from normal colons, kept at rest and stimulated at different frequencies, for 10 min. Clear rectangles, resting release ; hatched, net extra release during pelvic stimulation ; double hatched, net extra release during transmural stimulation. Each value is the mean of ten experiments.

course of long-lasting stimulation at relatively high frequency. These findings agree with similar observations made by others in different preparations (Birks & McIntosh ; 1961).

It is worthy of note, however, that the amount of acetylcholine associated with a given stimulation schedule did not appreciably change or slightly increase during the experiment, even if the periods of stimulation were repeated three or four times.

Effect of adrenaline, noradrenaline and sympathetic stimulation

Both adrenaline 1×10^{-7} g/ml. and noradrenaline 1×10^{-7} g/ml.–1×10^{-6} g/ml. were able to reduce the spasm of eserinized colons. On the other hand, only adrenaline 1×10^{-7} g/ml. and noradrenaline 1×10^{-6} g/ml. (but not 1×10^{-7} g/ml.) significantly reduced the acetylcholine release from unstimulated colons (Fig. 4). No effect on the acetylcholine output associated with transmural or pelvic stimulation

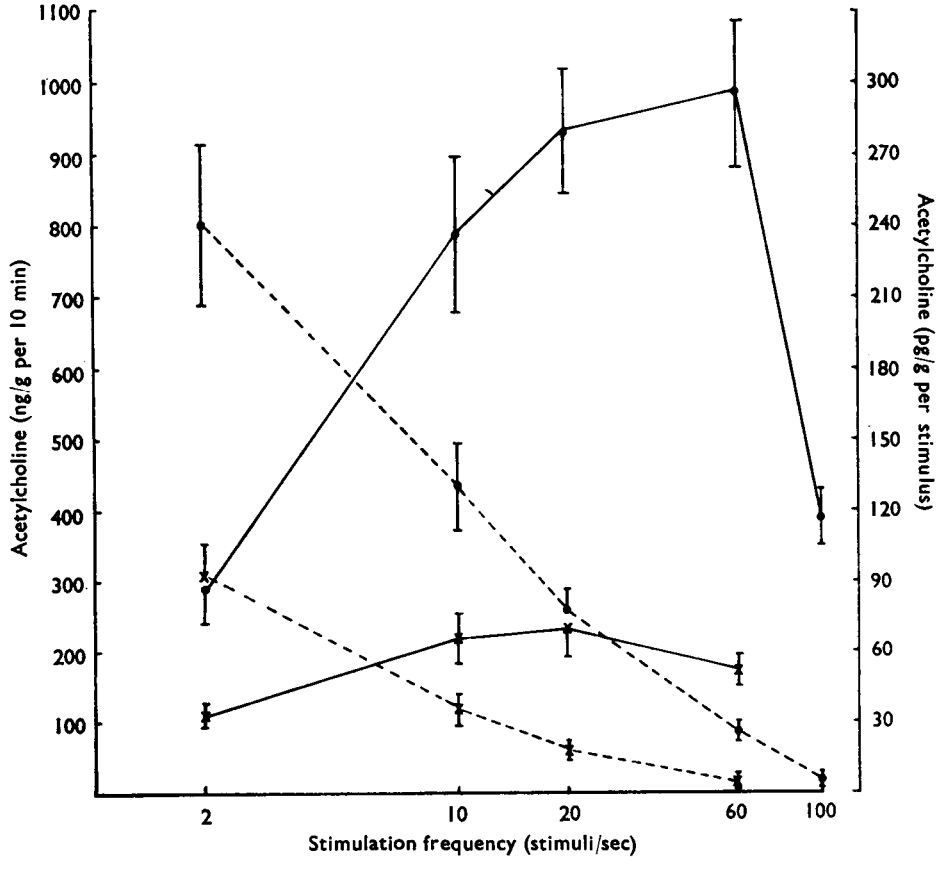

FIG. 2. Acetylcholine release from guinea-pig colons, expressed as output/min (left hand scale, continuous lines) and as output/stimulus (right hand scale, dashed lines) in response to transmural (●) and pelvic (×) stimulation at different frequencies lasting 10 min. The release was calculated as net extra release ; that is, by subtracting the output during a 10 min rest period. Each value is the mean of ten experiments (see Fig. 1). Vertical lines, S.E.

at 10/sec could be detected. After washing, the resting release approached pre-treatment levels. In the light of the suggestion put forward by some authors that catecholamines restrain the neuronal activity at low frequency, the same concentrations of adrenaline and noradrenaline were tested in preparations stimulated at 1/sec. As shown in Fig. 5, adrenaline 1×10^{-7} g/ml. and noradrenaline 1×10^{-6} g/ml. significantly reduced not only the resting release but also that associated with transmural stimulation.

Sympathetic stimulation at 50/sec for 10 min relaxed the colon but did not change the normal resting release; rather it was slightly increased, nor did it significantly reduce acetylcholine output during transmural or pelvic stimulation at 10/sec (Fig. 6). Conversely, sympathetic stimulation was able significantly to decrease acetylcholine release associated with pelvic and transmural stimulation at 1/sec, in agreement with the inhibitory effect displayed by catecholamines on the low-frequency neuronal activity. It remained unexplained, however, why sympathetic stimulation was unable to reduce the resting output. A possible reason could be that the reduction of acetylcholine outflow exerted by sympathetic stimulation was partly or completely overshadowed by the simultaneous acetylcholine output from some cholinergic fibres mixed with the stimulated sympathetic periarterial nerves. Thus the amount of acetylcholine released by perivascular stimulation was tested

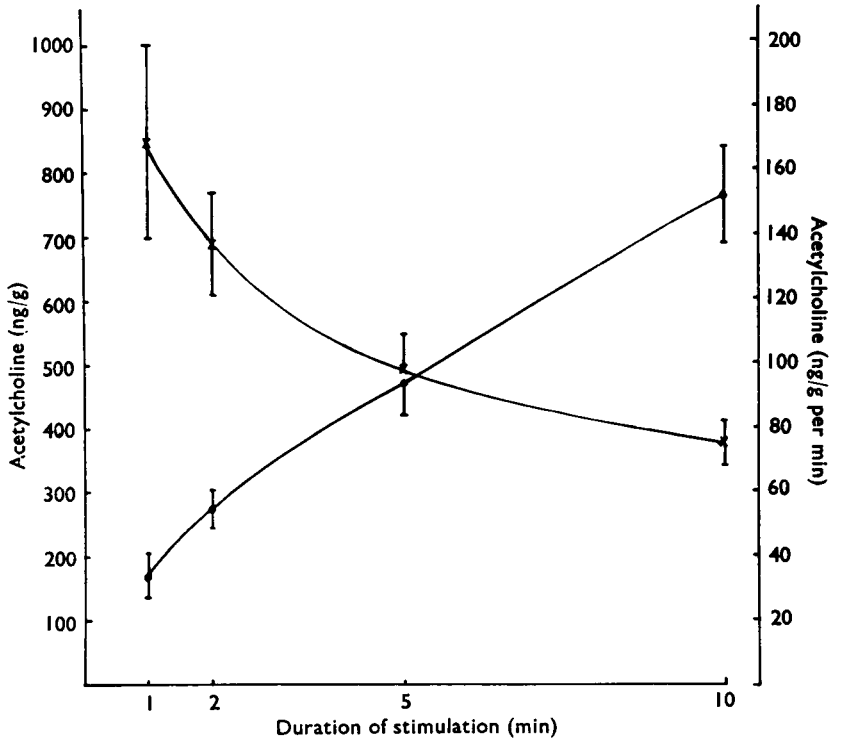

FIG. 3. Relationship between duration of transmural stimulation at 10/sec and acetylcholine released in the whole period (●——●) and acetylcholine released/min (×——×). The release was calculated as net extra release, by subtracting the output during the corresponding rest period. Every value is the mean of six experiments. Vertical lines, S.E.

in six preparations taken from guinea-pigs treated with reserpine. Reserpine 5 mg/kg was injected intraperitoneally 20 hr before they were killed. In these preparations the acetylcholine release at rest was 374 ± 23 (S.E.) ng/g per 10 min, but the release during sympathetic stimulation at 50/sec rose to 559 ± 60 (S.E.) ng/g per 10 min. The difference was statistically significant ($P<0.01$, Student's test for paired data).

In the colons of reserpine-treated animals, kept in normal Tyrode solution (without eserine), perivascular stimulation both at low and high frequency did not give rise to the usual relaxation; on the contrary, reversal was sometimes observed (Gillespie & MacKenna, 1961) scarcely affected by hexamethonium and abolished by atropine (Fig. 7).

These findings may explain why sympathetic stimulation did not "apparently" reduce acetylcholine release from the colon at rest: in fact the periarterial nerve fibres release enough acetylcholine (about 50% of background values) to compensate for the reduction exerted by sympathetic neurotransmitter on the spontaneous release from the intramural cholinergic structures. Such a statement, however, would require proof that most of the acetylcholine released was linked to nerve conducted activity. With this aim, the effect of adrenaline in the presence of hexamethonium and tetrodotoxin was examined.

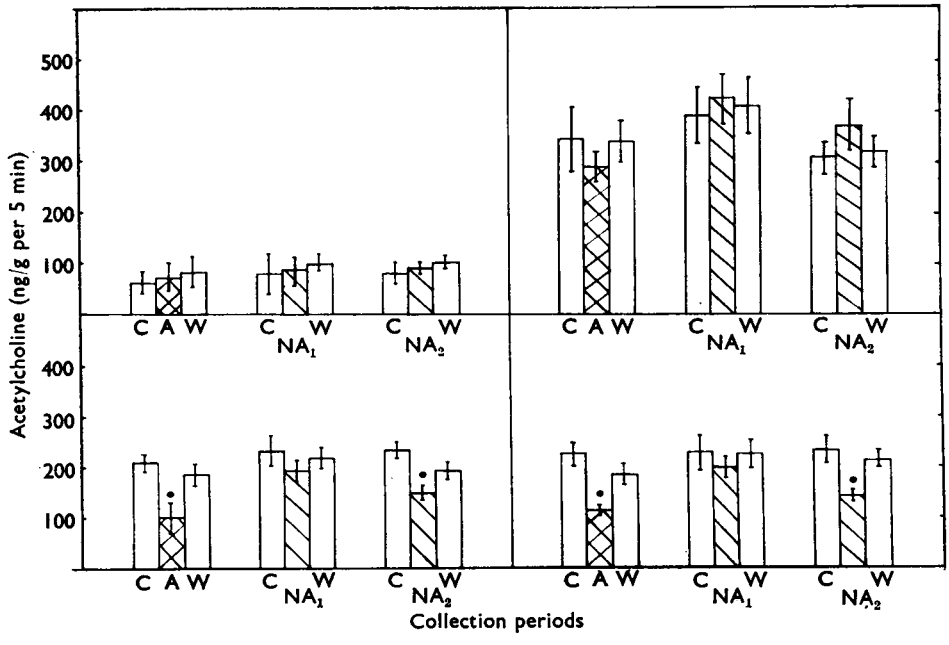

FIG. 4. Effect of adrenaline and noradrenaline on acetylcholine release (ng/g per 5 min $\pm$ S.E., vertical lines) from six groups of colons at rest and during pelvic (left) and transmural (right) stimulation at 10/sec for 5 min. Every value is the mean of six experiments. Lower figures, release at rest; upper figures, net extra release during stimulation. Clear rectangles, normal acetylcholine release before adding (C) and after washing out (W) catecholamines; double hatched, release in the presence of adrenaline 1×10^{-7} g/ml. (A); hatched, release in the presence of noradrenaline 1×10^{-7} g/ml. (NA_1) and 1×10^{-6} g/ml. (NA_2). ●, Significantly different from the control value ($P<0.01$, Student's test for paired data).

Effect of adrenaline in the presence of hexamethonium and tetrodotoxin

Hexamethonium 5×10^{-5} g/ml. – 2×10^{-4} g/ml. did not modify the spasm of the colon induced by eserine, but significantly reduced acetylcholine release both at rest and during transmural stimulation at 10/sec (Fig. 8). This finding strengthens the idea that: in the eserinized intestinal preparations at rest there is a background neuronal activity sustained by the efficiency of synaptic transmission (Zar, 1966). Moreover, during transmural stimulation, the intramural ganglion cells seem to undergo some kind of mutual excitation which is restrained by hexamethonium as suggested by the reduction exerted by the drug on the release of acetylcholine.

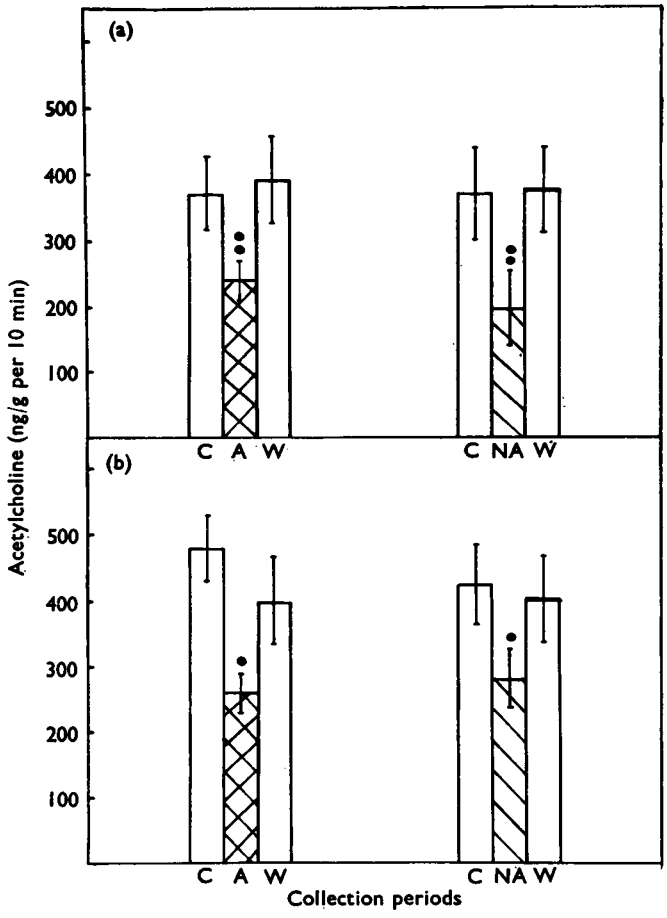

FIG. 5. Effect of adrenaline and noradrenaline on the acetylcholine release (ng/g per 10 min ±S.E., vertical lines) from two groups of six colons kept at rest and transmurally stimulated at 1/sec, for 10 min. (a) Net extra release during stimulation; (b) clear rectangles, acetylcholine release before adding (C) and after washing out (W) catecholamines; double hatched, release in the presence of adrenaline 1×10^{-7} g/ml. (A); hatched, release in the presence of noradrenaline 1×10^{-6} g/ml. (NA). ●, Significantly different from the control value ($P<0{\cdot}01$, Student's test for paired data); ●●, $P<0{\cdot}001$.

Although synaptic transmission was impaired by hexamethonium, adrenaline was still able further to reduce the release of acetylcholine from unstimulated colons. A possible explanation for the additional effect exerted by adrenaline in colons pretreated with hexamethonium is found in the observations of De Groat & Volle (1964). These authors stated that hexamethonium did not affect the ganglionic repetitive firing caused by inhibitors of acetylcholinesterase, while catecholamines were able to inhibit it. Moreover, the possibility that adrenaline acts also on cholinergic nerve-endings cannot be ruled out, in view of the observations of Paton & Thompson (1953) that the amine reduced acetylcholine release from the eserinized superior cervical ganglion.

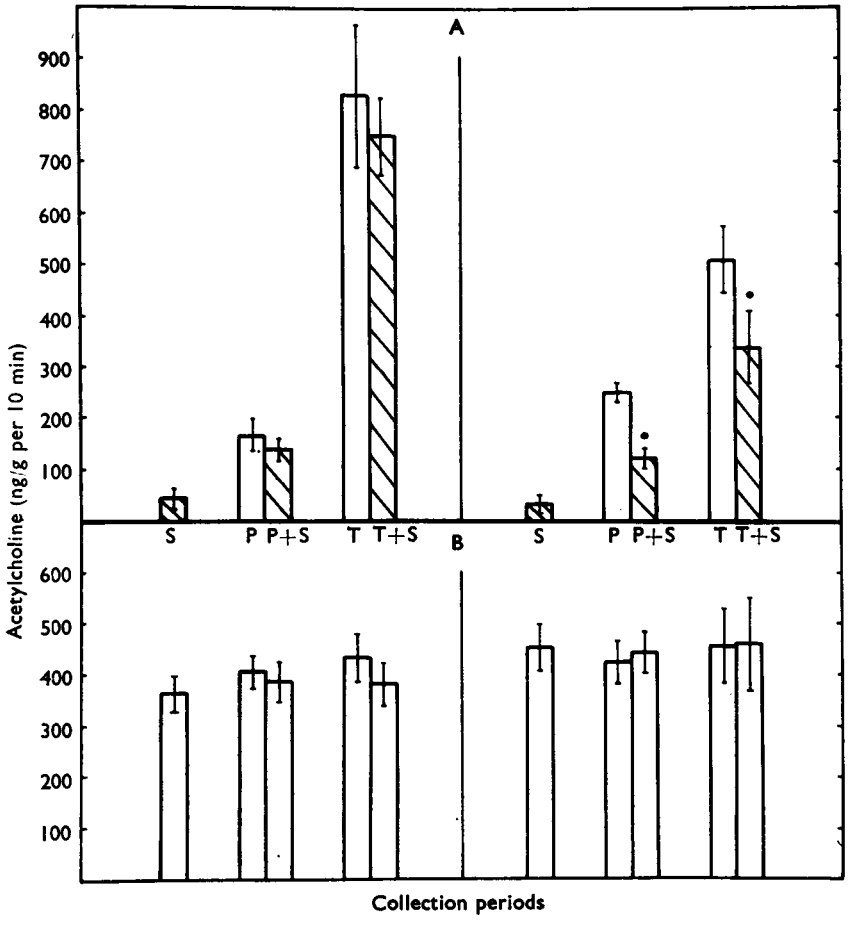

FIG. 6. Effect of sympathetic stimulation (50/sec, for 10 min) alone or associated with pelvic and transmural stimulation at 10/sec (left) and at 1/sec (right) on acetylcholine release (ng/g per 10 min ± S.E., vertical lines) from two groups of ten colons. A, Clear rectangles, net extra release during pelvic (P) and transmural (T) stimulation alone; hatched, net extra release during sympathetic stimulation at 50/sec alone (S) or associated with pelvic (P + S) and transmural (T + S) stimulation. B, Acetylcholine release in the corresponding periods of 10 min rest. ●, Significantly different from the control value (P or T stimulation alone), $P<0.01$, Student's test for paired data.

Further information was obtained from colons treated with tetrodotoxin. It is well known that the drug selectively blocks axonal conduction, leaving unaltered the spontaneous neurosecretion of nerve-endings (Kao, 1966).

In our experimental conditions, tetrodotoxin 1×10^{-7} g/ml. was able, within 45 min, to abolish the relaxation of the eserinized colons to transmural or sympathetic stimulation, suggesting complete axonal blockade. At this time, the spasm of the preparations was unchanged or only slightly reduced, but the acetylcholine release fell to about 10% of the control value.

The small release at rest that remained, was unaffected by adrenaline up to 1×10^{-6} g/ml. (Fig. 9). In eserine-treated colons, therefore, (1) the spasm is caused by a largely supramaximal amount of acetylcholine, unaffected by tetrodotoxin; (2) the normal release at rest is almost certainly linked to nerve conducted activity; (3) only the acetylcholine release associated with nerve conducted activity is catecholamine-sensitive.

The acetylcholine released in preparations treated with tetrodotoxin probably originates from spontaneous neurosecretion by the parasympathetic nerve endings, although the existence of an extraneuronal pool insensitive to catecholamines and tetrodotoxin cannot be excluded.

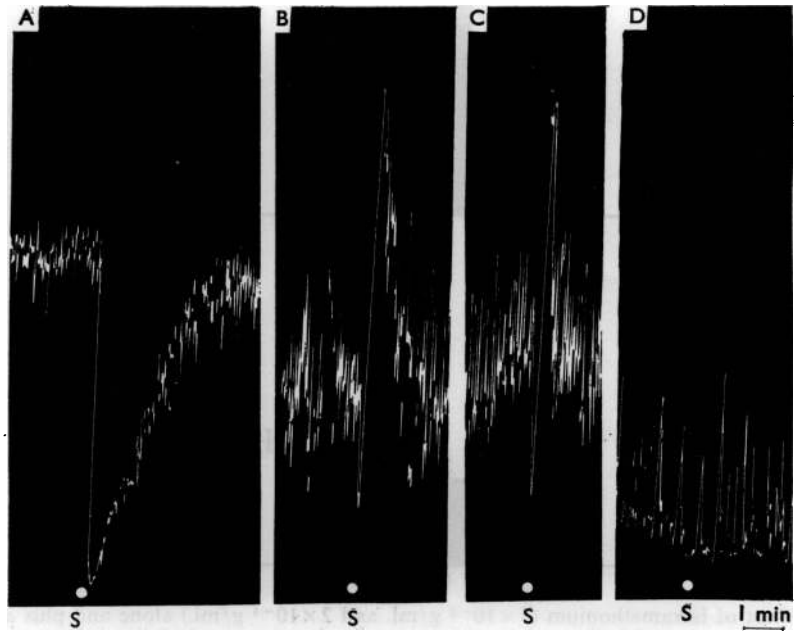

FIG. 7. A, Response of guinea-pig distal colon, kept in normal Tyrode solution at 35° C, to sympathetic stimulation at 50/sec (S) lasting 30 sec; B, response of a colon taken from reserpine-pretreated guinea-pig (5 mg/kg intraperitoneally 20 hr before death); C, as in B, in the presence of hexamethonium 5×10^{-5} g/ml.; D, as in B, in the presence of atropine 1×10^{-7} g/ml.

Tissue acetylcholine and cholineacetyltransferase activity

In order to establish whether the inhibitory effect exerted by catecholamines on acetylcholine release depended on some disturbances in synthesis and storage processes, the acetylcholine content of some colons was estimated. The acetylcholine content of normal, unstimulated colons kept for 2 hr in oxygenated Tyrode solution at 35° C was about 8 μg/g. The value was slightly, but not significantly higher in the presence of eserine 1×10^{-5} g/ml. ; adrenaline was without effect in both normal and eserinized preparations (see Table 2). Thus the reduction in release cannot be

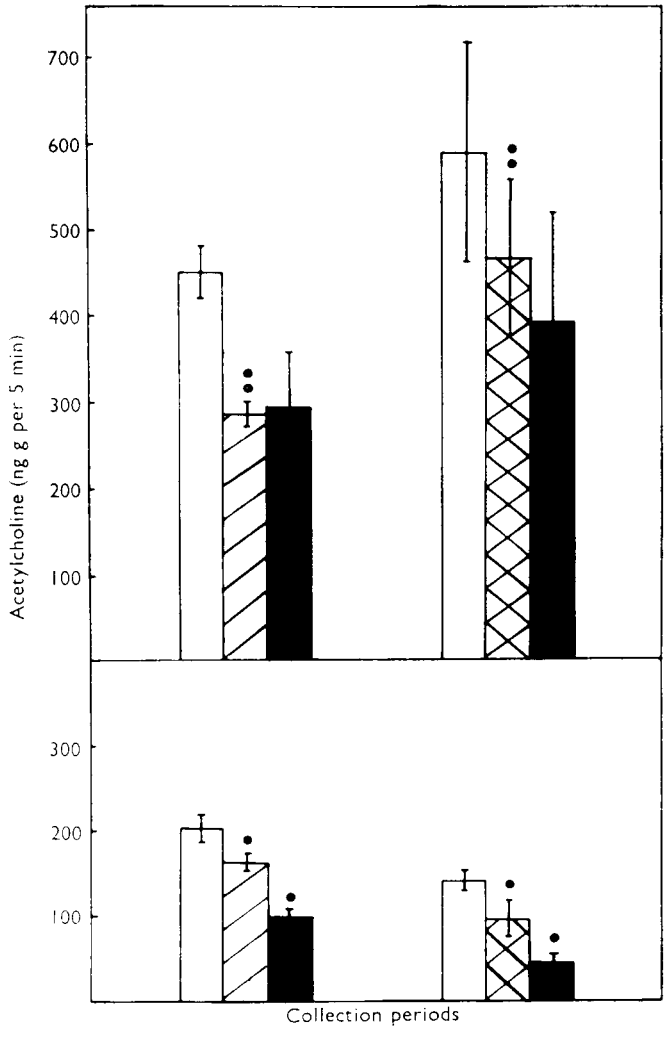

FIG. 8. Effect of hexamethonium (5×10^{-5} g/ml. and 2×10^{-4} g/ml.) alone and plus adrenaline 1×10^{-7} g/ml. on acetylcholine release (ng/g per 5 min ± S.E., vertical lines) from two groups of six colons kept at rest and transmurally stimulated at 10/sec for 5 min. Lower rectangles, release at rest ; upper rectangles, net extra release during stimulation. Clear rectangles, normal acetylcholine release ; hatched, release in the presence of hexamethonium 5×10^{-5} g/ml. ; double hatched, hexamethonium 2×10^{-4} g/ml. ; black, hexamethonium plus adrenaline 1×10^{-7} g/ml. ●, Significantly different from the control value (preceding period), $P<0.02$ (Student's test for paired data) ; ●●, $P<0.01$.

accounted for by the reduction in available acetylcholine. The amount of acetylcholine stored in the colon is very high and the inhibition of esterases does not change the figure as in other tissues, for example, the superior cervical ganglion where an accumulation of acetylcholine becomes quite evident after eserine (Birks & McIntosh, 1961). Our results agree with those of Gilfrich, Röttcher & Straub (1966), who found that eserine increases the acetylcholine content of guinea-pig ileum by only about 10%.

In contrast to the high acetylcholine content, the cholineacetyltransferase activity is relatively low. The synthesizing power of six freshly excised colons was 66 μg/g fresh tissue per hr ± 7 (S.E.). This value agrees well with the findings of Feldberg & Lin (1950) but is much lower than that found in guinea-pig caudate nucleus, the acetylcholine content of which approaches that of the colon (Beani, Bianchi &

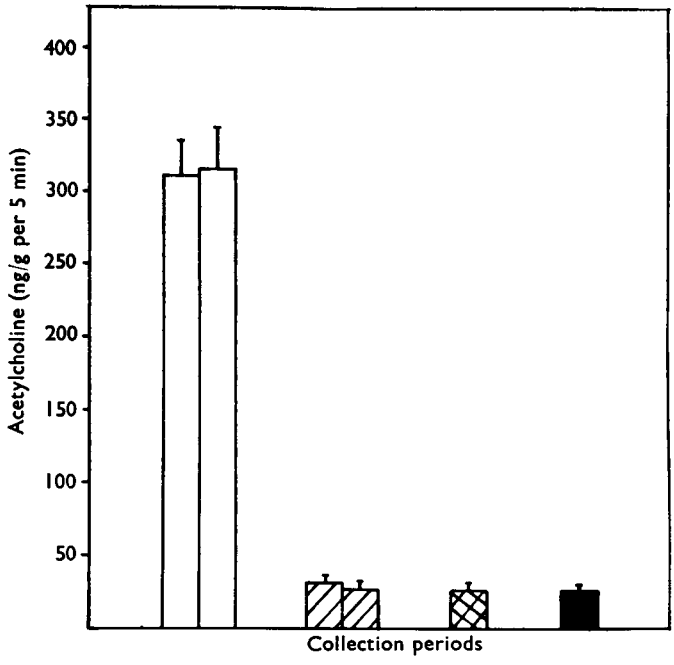

FIG. 9. Effect of tetrodotoxin 1×10^{-7} g/ml. on acetylcholine release (ng/g per 5 min $\pm$ S.E., vertical lines) from one group of six guinea-pig colons kept at rest. Clear rectangles, normal acetylcholine release; hatched, release 45 min or more after adding tetrodotoxin; double hatched, release in the presence of tetrodotoxin plus adrenaline 1×10^{-7} g/ml.; black, release in the presence of tetrodotoxin plus adrenaline 1×10^{-6} g/ml.

TABLE 2. *Acetylcholine content of guinea-pig distal colon kept for 2 hr in normal Tyrode solution and in Tyrode plus eserine* 1×10^{-5} *g/ml. in the presence, or absence, of adrenaline* 1×10^{-7} *g/ml.*

Experimental conditions	Acetylcholine (μg/g of fresh tissue $\pm$ S.E.)	
	Normal Tyrode solution	Tyrode plus eserine 1×10^{-5} g/ml.
Controls	8·82±0·65	9·32±0·45
Adrenaline	8·73±0·66	9·06±0·49

Temperature 35° C. Each value represents the mean of six colons.

Megazzini, 1964). Thus the ratio between synthesizing activity and acetylcholine content in the guinea-pig colon is lower than that found in other tissues of the same animal.

Nevertheless, the cholinoacetyltransferase activity is enough to ensure the high content and release observed.

Discussion

The neuronal origin of intestinal acetylcholine seems at present to be firmly established (Paton & Zar, 1968). A great deal of experimental evidence suggests that the intramural plexuses undergo activation by mechanical or electrical stimulation of the parietal wall, so that acetylcholine release increases (Hayama & Ikeda, 1959 ; Kažić & Varagić, 1968 ; Paton, 1957). Consequently the effect exerted on acetylcholine release by many drugs and experimental conditions (Schaumann, 1958 ; Harry, 1962 ; Johnson, 1963 ; Brownlee & Johnson, 1965 ; Takagi & Takayanagi, 1965 ; Cox & Weinstock, 1966 ; De La Lande & Porter, 1967) may be explained either by changes in the traffic of impulses travelling through the neuronal network or by direct effects on the neurosecretory processes of the cholinergic nerve endings. No doubt, catecholamines reduce acetylcholine release from intestinal preparations at rest or stimulated at low frequency, as shown by Schaumann (1958) and confirmed by Vizi (1968), Kosterlitz & Lydon (1968) and ourselves. Such findings are not, however, an irrefutable proof of the physiological restraining action exerted by the sympathetic system on cholinergic structures. In this respect, more convincing arguments are offered by the functional investigations carried out by Jansson & Martinson (1966) and Kewenter (1965), even if the criticism put forward by Gershon (1967) is accepted.

Our results strengthen the idea that the sympathetic nerves actually control the activity of intramural plexuses, as judged by the effect exerted on acetylcholine release when they are stimulated. The lack of such evidence in Gershon's (1967) experiments must be referred to the simultaneous release of acetylcholine in amounts able to mask the expected effect. The origin of acetylcholine released by stimulating the periarterial nerves is uncertain. There are no valid means of determining whether acetylcholine originates from true cholinergic fibres mixed with sympathetic ones (Boyd, Gillespie & MacKenna, 1962) or from the sympathetic nerve-endings themselves (Burn & Rand, 1965). Whatever the right explanation may be, the restraining effect exerted by sympathetic nerves on the cholinergic intramural structures is established. In view of this statement, caution is required in evaluating results obtained by stimulating the intestinal wall. In this intriguing condition, in fact, both cholinergic and sympathetic nerves are simultaneously affected so that : (1) the effect of the additional stimulation of periarterial nerves, even if at higher frequencies, is minimized and (2) the relationship between stimulation rate and acetylcholine release might have been different from that shown in Fig. 2, if the sympathetic intramural fibres had been functionally excluded. Accordingly, Vizi (1968) found that pretreatment with reserpine increases acetylcholine release in the guinea-pig ileum stimulated at low frequency. The intimate mechanism of the inhibition exerted by catecholamines on the cholinergic structures still needs to be clarified. Taking into account the results of Paton and Thompson (1953), a direct effect on the cholinergic nerve endings may be postulated. Catecholamines seem also to exert some action on the post-activation membrane potential changes of

ganglion cells (De Groat & Volle, 1964) by abolishing the phase of hyper-excitability responsible for the after discharge. In both instances a " stabilizing " effect on the transmembrane potential could suppress the repetitive firing of cell bodies and of nerve endings. Unfortunately these inferences are drawn from experiments far removed from the physiological conditions—that is, in the presence of anticholinesterase agents.

The complex effect of such drugs is well known (Bell, 1966 ; Takeshige & Volle, 1962 ; Standaert & Riker, 1967) and one cannot rule out a possible action of catecholamines on the anomalous functional feature, such as repetitive firing, caused at the synapses and at the nerve endings. Therefore, although our results further support the hypothesis of sympathetic control on the cholinergic neurones, a more direct proof of this effect is advisable.

The question arises whether the sympathetic system modifies gastrointestinal motility only by restraining the intramural cholinergic activity. It cannot be denied that: (1) the normal tone and motility of isolated colon is scarcely affected by tetrodotoxin (Kao, 1966) thus the simple suppression of nerve-conducted activity does not change the background functional state ; (2) sympathetic stimulation actually inhibits tone and motility even in atropinized preparations (Gershon, 1967) so a direct effect of released catecholamines on the smooth muscle cells must be accepted.

Our opinion on the actual part played by the sympathetic system lies between the different attitudes taken by Gershon (1967) and by Kewenter (1965) and Jansson & Martinson (1966): the extrinsic nervous inhibitory system probably employs the less complicated means of producing its effects: in other words, direct inhibition of smooth muscle, paralleled by the simultaneous inhibition of the excitatory cholinergic activity.

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